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THE FROG

A LABORATORY GUIDE

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THE FROG

A Laboratory Guide

BY

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PREFACE

The frog needs no introduction to the zoölogical laboratory, where it has assisted many generations of students in acquiring their first knowledge of vertebrate anatomy. As Professor Holmes writes in his Biology of the Frog, "They are nice clean animals, easy to dissect and admirably fitted for physiological experimentation."

In most localities they are easily collected and may be kept in the laboratory alive. All dealers in biological supplies handle them both alive and preserved. Natural sources of supply are being augmented constantly by the development of frog-hatcheries to supply the commercial demand.

The literature dealing with the frog is voluminous. No attempt is made in this guide to indicate more than a few of the books dealing with the anatomy of the frog which may be useful in supplementing this brief sketch.

It has been held by some that the function of a laboratory guide is to *ask* the student what he finds instead of *telling* him what he is to find, but, in the experience of many teachers, the "problem" type of laboratory manual is apt to waste the time of both instructor and student, and wear out the patience of both. It is the writer's opinion that the needs of the beginning anatomist are best served by presenting him with a straight-forward account of what he is to find in the specimen he is dissecting. If he encounters an anomaly or discovers the writer in a slip, he may be referred to larger manuals and to original contributions, and taste for himself the joys and hardships of research.

From time to time directions for the preparation of drawings are inserted. It is the writer's hope that these drawings will be used by the student as a means to the correlation of the anatomical details he has verified, rather than by the instructor as a method of judging the proficiency of the student. Frequent oral quizzes and demonstrations help make the laboratory a dissecting-room, instead of a drafting-room, as is too often the case.

Following the account of the anatomy of the adult frog, there will be found sections on the histology and embryology of this form. It is hoped that these will be useful in those laboratories where the frog plays the central rôle in the beginning course.

The terminology employed has been carefully compared with that of Holmes, which is followed as a general rule.

In conclusion, the writer wishes to express his appreciation of the kindness of his colleague, Dr. A. R. Cahn, in reading the manuscript and making many helpful suggestions.

Waldo Shumway.

Urbana, Ill.
April, 1928.

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THE FROG

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A LABORATORY GUIDE

INTRODUCTION

The frog is a tailless amphibian vertebrate. Its exact position in the system of classification is indicated below:

Phylum: Chordata,
Sub-phylum: Vertebrata,
Super-class: Tetrapoda,
Class: Amphibia,
Sub-class: Lissamphibia,
Order: Anura,
Family: Ranidæ,
Genus: Rana.

One hundred and ninety-five species of this genus[1] have been described, twenty-one of which are native to North America. Of the latter the most common species is *Rana pipiens*, the leopard frog, upon the anatomy of which these directions are based. The largest species is the bullfrog, *Rana catesbiana*, and for this reason a more desirable form for laboratory work. The dissections here described have been repeated upon the bullfrog, and any striking divergences are noted. The latest key to the American species is contained in Boulenger's *Monograph of the American Frogs of the Genus Rana.* It should be noted that this author uses the specific name *halecina* instead of the

[1] The tree frog and the toad belong to different genera although members of the same order.

more familiar *pipiens*. Excellent descriptions and illustrations are to be found in Miss Dickerson's *Frog Book*.

The following characters of the frog are peculiarly amphibian. The skin is glandular and without an exoskeleton. The skull is autostylic and its articulation with the vertebral column is by means of paired condyles on the exoccipitals. The basioccipital and supraoccipitals are lacking as well as the basisphenoid. The squamosals and parasphenoid are well-developed. The kidney is a mesonephros and there is present an urinary bladder. The male mesonephric duct functions as a ureter and gonoduct. In the female the mesonephric duct carries only excretory products, and an extra tube, the oviduct, is present to transport the products of the gonads. The heart has two auricles and one ventricle. Three aortic arches persist. The cardinal veins have been largely supplanted by a postcaval vein. Both a renoportal and an hepatoportal system are present. There is a comprehensive lymphatic system. The red blood corpuscles are large and oval with a prominent nucleus. The brain possesses a small cerebellum. Ten cranial nerves are found. The olfactory sacs communicate with the mouth by means of posterior nares. There is no outer ear. The tympanic membrane is attached to a columella which extends across the middle ear to join a cartilage which has been considered the stapes, lying in the fenestra ovalis.

Gaupp's revision of Ecker and Wiedersheim's *Die Anatomie des Frosches* remains the most comprehensive memoir on the anatomy of the frog. *The Biology of the Frog* by Holmes is a convenient compendium with especial regard to *R. pipiens*.

The student must bear in mind that the frog is not a good example of the generalized amphibian type. Necturus, one of the tailed amphibia (see Adams, L. A., *Necturus, a*

Laboratory Manual) is preferable for this purpose. The Anura or tailless amphibia are characterized not only by the lack of a tail, but by the short broad trunk and the fact that the hind legs are much larger than the fore legs. The vertebræ are generally procoelous and the caudal vertebrae are fused into a single bone, the urostyle. No ribs are present. The skull shows certain peculiarities such as the fusion of the frontals and parietals, and the presence of a columella. A clavicle is present in the shoulder girdle, and the ilium of the pelvic girdle is greatly elongated. The radius and ulna are fused into a single bone, as are the tibia and fibula. In the respiratory system, no distinct trachea is found. The fifth aortic arch is absent in the adult. The kidney is shorter than in the Caudata.

The tissues of the frog are well adapted to serve as an introduction to histology. The cells are large while the organs are small enough so that sections through entire organs can be viewed within the field of the low power objective. Examples of all common vertebrate tissues may be found.

The embryology of the frog presents the following points of interest. Fertilization is external. The egg is fairly large and segmentation is quite regular through the first cleavages. Hemisections through the blastula and gastrula are instructive at low magnifications. The young hatch as larvæ (tadpoles) with first external and then internal gills. The large tadpole of the bullfrog is easily dissected. Stages in metamorphosis may be demonstrated.

Dissection is a technique which requires, above all things, both care and patience. Follow directions carefully. Never cut anything away without knowing what you are cutting and what you are looking for. If you get in trouble, stop, wash the specimen under the tap and make a fresh start. Keep your dissecting instruments clean and sharp. Clear

away the débris as it accumulates and put everything in its place at the end of the hour. Remember that accurate observation is as important as careful dissection. Do not depend on your memory, but make working sketches as you dissect. Do not neglect your ruler; a few accurate measurements will make your final drawing more valuable than hours of "finishing." Label every structure appearing on the drawing.

Learn the following descriptive adjectives: *dorsal,* with reference to the back; *ventral,* with reference to the belly; *cephalic,* with reference to the head (= anterior); *caudal,* with reference to the tail (= posterior); *mesial,* with reference to the mid-line; *dextral,* with reference to the right side of the animal itself; *sinistral,* with reference to the left side; *proximal,* referring to the part nearest the mid-line of the body; and *distal,* referring to the part farthest away from the mid-line. Any of these adjectives may become adverbs by the substitution of the suffix *-ad* for *-al,* e.g., *dorsad* means in the direction of, or toward, the back.

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SECTION I. EXTERNAL CHARACTERS

Place the frog on your dissecting pan, dorsal side up, and locate the following structures:

1. The **head** is roughly triangular in shape.
2. The **mouth** is large, terminal, and probably tightly closed.
3. The **eyes** are large and bulging. In the living specimen a central **pupil**, black and oval, surrounded by a bright, colored **iris**, can be distinguished.
4. There are two **eyelids**, upper and lower (the latter with an attached **nictitating membrane** which can with difficulty be demonstrated).
5. The **brow spot**, a light spot between the eyes, is the vestige of the pineal eye, and embryologically connected with the epiphysis (176).
6. Caudal to the eyes is the **tympanic membrane** (tympanum) of the ear, in the center of which is a slight projection caused by the columella (165), one of the ear bones. What significance do you find in its position on the tympanum?
7. At the cephalic end, the **external (anterior) nares**, or nostrils, are found.
8. The **trunk** bears two pairs of appendages.
9. The **fore limb** consists of **upper arm, lower (fore) arm**, and **hand**; the latter has four **fingers** and a vestigial **thumb**. The first and second fingers have three **joints** each, the others four.
10. The **hind limb** consists of **thigh, shank,** and **foot;** the latter has five **toes** and a rudimentary sixth

toe, the **prehallux**, on the inner side. The first two toes each have three **joints**, the next two have four, and the fifth, five. The toes are joined by a **web**. How far does it extend on each toe?

11. The **dermal plicæ** are folds extending caudad from the eyes. How many are there, and how far can you trace them on your specimen?
12. The **vent** or cloacal aperture is mesial and dorsal to the hind legs.

Study the pigmentation. What colors are found, and where? What is the size, shape, and pattern of the spots? Both pigmentation and pattern are especially important in *R. pipiens*.

Make the following measurements, in millimeters:

a) from snout to vent;
b) head, length;
c) head, width;
d) snout;
e) eye;
f) width between eyes;
g) tympanum;
h) forelimb, and subdivisions;
i) hindlimb, and subdivisions.

Drawing 1. Draw the dorsal view, the head towards the upper edge of the sheet, natural size (x1).[1]

[1] Bullfrog (x½).

SECTION II. MOUTH AND PHARYNX

Open the mouth of the frog as widely as possible and locate the following structures, commencing with the roof.

13. The **lips** are thin and fleshy.
14. The **maxillary teeth** are on the mesial edge of the upper jaw; they are fine, conical, and closely set. Note their extent.
15. The **sulcus marginalis** is a groove into which the lower jaw fits closely.
16. The **vomerine teeth** are in two groups at the cephalic end of the roof.
17. The **posterior (internal nares)** are lateral to the vomerine teeth. *Probe these from the external nares* (7) *with a bristle.*
18. At the caudal end of the cavity close to the angle of the jaws, locate the openings of the **Eustachian (auditory) tubes.**
19. The portion of the cavity into which these open is the **pharynx.**
20. The pharynx communicates with the digestive tract by the **œsophagus**, a transverse slit in the mesial line, between the Eustachian tubes.
21. Cephalad to the œsophagus and on the ventral surface of the pharynx is a cephalo-caudal slit, the **glottis**, opening into the larynx (in reality, laryngeo-trachea) (60).
22. The glottis is surrounded by two prominences caused by the **arytenoid cartilages** of the larynx.

23. Lateral to the glottis, near the angle of the jaws, in positions corresponding to the Eustachian tubes, may be found the apertures of the **vocal sacs** if the specimen be a male. These may be probed or demonstrated with the blow pipe in a fresh specimen.
24. The **tongue**, large and fleshy, is wholly in the mouth cavity. Examine the peculiar method of its attachment.

Drawing 2. Draw (x2) a full front view of the mouth and pharynx, dorsal side uppermost, the jaws opened wide so as to show the buccal structures.

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SECTION III. THE VISCERAL CAVITY

1. The Viscera Undisturbed

Place the frog on the dissecting board, ventral side up, and with the head away from you. Holding the skin away from the underlying body wall with forceps, make an incision through the skin only from the pelvic girdle to the lower jaw. Carefully lift the skin with forceps and note the following.

25. Subcutaneous **lymph spaces** are separated by the points of attachment between the body wall and the skin. In life they are filled with a colorless fluid, the **lymph.**
26. On the inner surface of the skin are the **cutaneous arteries and veins**, by means of which cutaneous respiration is performed. Make out the points at which these enter the underlying tissues.
27. There is an **abdominal vein**, showing through the muscles of the body wall on the mid-line.

Cut through the body wall slightly to one side of the abdominal vein. Lift the body wall away from the underlying organs with your forceps and take care not to cut across any blood vessels. Use heavy scissors to cut across the pectoral girdle. Complete this incision to the same limits attained in the skin incision. Note the following structures without disturbing their position.

28. At the cephalic end is the **heart**, underneath the pectoral girdle.

29. It lies in the **pericardial cavity**, which is surrounded by
30. a thin glistening membrane, the **pericardium**, which separates the pericardial cavity from
31. the **abdominal cavity**, or **cœlome**, which contains the remaining viscera.
32. This cavity is lined by a membrane which continues over the viscera and is called the **peritoneum**.
33. The most conspicuous organ of the viscera is the **liver**, a large, dark, reddish brown gland just caudal to the heart. Between its two lobes
34. the dark green **gall bladder** may sometimes be seen.
35. Caudal to the liver and partially concealed by its lobes is the large, white, U-shaped **stomach**.
36. Sometimes the **pancreas**, a yellow, flat, and elongated gland with irregular lobes, may be seen at the point where the stomach joins the
37. **duodenum**, or cephalic portion of the intestine, not always visible when the organs are undisturbed.
38. The **urinary bladder** is very large and has a thin wall. It is best demonstrated in a fresh specimen by inserting a blowpipe into the vent and distending it with air.
39. The large **ovaries** filled with black and white eggs are the most prominent structures visible in a mature female at certain seasons of the year.

Drawing 3. Make a sketch (x1) of the viscera as they lie in place without disturbing them. If your specimen is a mature female, turn to page 18 and work out the structure of the female reproductive system before continuing.

2. The Digestive Organs

Push a needle handle or pipette into the opening of the œsophagus (20), *if possible as far as the stomach* (35), *and raise the liver, without cutting anything. Trace the entire digestive system, noting that it is essentially a tube, or canal with an anterior and posterior aperture, and having two large glands, the liver and pancreas, emptying into it. Locate the following structures which have not been previously noted*:

40. The **cardiac end** of the stomach is that into which the œsophagus enters.
41. The posterior or **pyloric end** is where the stomach empties into the duodenum (37).
42. The **bile duct** can be demonstrated in a fresh specimen by squeezing the gall bladder (34) gently. From the gall bladder it empties into the duodenum a short distance below the pyloric end of the stomach. It is formed by the union of the
43. **hepatic ducts**, leading from the lobes of the liver and
44. is connected with the gall bladder by the **cystic ducts.**
45. The **ducts** of the pancreas (36) open into the bile duct but are not easily demonstrated.
46. The duodenum continues caudal to the point where the bile duct enters as the small intestine or **ileum.**
47. The ileum terminates in the large intestine or **rectum,** which enters
48. the **cloaca**, the external aperture of which is the vent (12).

3. The Mesenteries

You should have noted by this time that the viscera are attached to the body wall as well as to each other by delicate membranes. These are the mesenteries. Locate the following membranes.

49. The **mesogaster** extends from the stomach to the dorsal body wall.
50. The **mesoduodenum** stretches from the duodenum to the dorsal body wall.
51. The **mesentery proper** is the membrane from the small intestine to the dorsal body wall.
52. The **mesorectum** is a corresponding structure from the rectum to the dorsal body wall.
53. The **gastro-hepatic omentum** stretches from the stomach to the liver.
54. The **gastro-duodenal omentum** extends from the stomach to the duodenum.
55. The **hepato-duodenal omentum** connects the liver and the duodenum.
56. The **ventral ligament (mesohepar)** is a portion of the embryonic ventral mesentery from the liver to the ventral body wall.
57. The **spleen** is a small dark red hæmolymph gland in the mesentery proper.

Drawing 4. Make a semi-diagrammatic sketch of the digestive system (x1), including the mesenteries.

4. The Respiratory System

The larval frog, or tadpole, breathes by means of gills. The adult possesses functional lungs, but also respires through the skin by means of the cutaneous veins and arteries (26).

Lift the liver and observe:

58. The **lungs** are brown, more or less shriveled structures at the cephalic end of the abdominal cavity. In the fresh specimen they may be demonstrated by inserting the blow pipe into the glottis (21) and inflating them. The blood vessels then apparent are
59. the **pulmonary arteries** and **veins.**
60. The tube leading from the glottis to the lungs is the **larynx.** Probe it.

Add the lungs to Drawing 4, and show the larynx by dotted lines.

5. The Visceral Arteries

The digestive system must be removed in order to see the underlying structures more clearly. But before doing this it is advisable to identify the arteries and veins associated with these structures. In an uninjected specimen the arteries, carrying blood away from the heart, are usually empty and colorless, while the veins, which carry blood toward the heart, are congested and therefore reddish brown in color. In singly injected specimens the arteries are filled with a colored starch mass, while the veins are filled with blood. In doubly injected specimens, the portal systems (76-80) are injected with a contrasting color mass. Triply injected frogs have a mass of a third color injected into the remaining veins.

Identify the following arteries without cutting anything.

61. Tip the liver to one side and lift the stomach, turning it to your left, and in the mesentery proper (51) in front of the spleen (57) observe the **cœliaco-mesenteric artery.** It arises from the dorsal aorta (139) and soon divides into two branches (62 and 69).

62. The cephalic branch is the **cœliac artery.** It divides into two branches (63 and 64).
63. The **left gastric artery** runs along the mesogaster (49) sending numerous branches to the left side of the stomach.
64. The **gastro-hepatic artery** runs from the cœliac, after the left gastric has been given off, along the mesoduodenum (50) and divides into two branches (65 and 67).
65. The **right gastric artery** runs from the gastro-hepatic along the gastroduodenal omentum (54) to the right side of the stomach which it supplies by many small branches. It gives off in passing
66. a small **gastro-pancreatic artery.**
67. The **hepatic artery**, a branch of the gastro-hepatic (64), runs along the hepato-duodenal omentum to the liver, which it supplies with arterial blood.
68. A small **hepato-pancreatic artery** is given off by the hepatic as it passes the pancreas.
69. The **anterior mesenteric artery** is the second branch of the cœliaco-mesenteric (61). It gives rise to two branches (70 and 72).
70. The **splenic artery** is a very short branch from the anterior mesenteric (69) arising soon after it leaves the cœliaco-mesenteric (61). It supplies the spleen.
71. The main portion of the anterior mesenteric is the **intestinal artery**, running to all portions of the small intestine along the mesentery proper. Some branches course through the mesorectum to supply the rectum. These are called the **hæmorrhoidal arteries.**

Drawing 5. Make a working sketch of the visceral arteries.

6. The Visceral Veins

Passing from the organs to which you just traced the visceral arteries, trace the visceral veins.

72. From the rectum and small intestine, numerous small vessels converge in the mesentery proper to form the **intestinal vein.**
73. The **splenic vein** joins one of the large tributaries of the intestinal vein which collects blood from the rectum.
74. The **gastroduodenal vein** carries blood from the duodenum and pyloric end of the stomach into the intestinal vein, passing through the gastro-duodenal omentum.
75. The **gastric veins** from the right and left sides of the stomach join with the intestinal vein as it courses along the hepato-duodenal omentum, to form
76. the **hepato-portal vein**, which enters the liver. It is largely obscured by the pancreas through which it passes at this time. From this gland it receives numerous small **pancreatic veins**, too small for practical demonstration.
77. From the point where the hepato-portal vein enters the liver, trace back a small vein, the **ramus descendens** of the abdominal vein, which you have already identified (27). Note that two other **rami** are given off from the abdominal vein at this point, which enter the right and left lobes of the liver.
78. Trace the abdominal vein back along the ventral surface of the body wall until you find its origin, the union of two **pelvic veins**, one from each leg.
79. At the point of union of the pelvic veins locate a small vein from the bladder, the **vesical vein.**

80. Toward the cephalic end, at the point where the abdominal vein leaves the body wall, it is joined by a small vein from the heart, the **cardiac vein.**

Drawing 6. Make a working sketch of the hepato-portal system, which is composed of the portal and abdominal veins and their tributaries.

SECTION IV. THE UROGENITAL ORGANS

The urogenital system is a complex of two systems, the excretory (nephric) system and the reproductive (genital) system. The excretory system is composed of the excretory glands or kidneys (mesonephroi in the frog), their ducts, the ureters (mesonephric ducts in the frog), and a storage sac, the excretory bladder. The reproductive system is composed of the genital glands or gonads (ovaries or testes), and their gonoducts (mesonephric duct in the male, oviduct in the female). Both open into the cloaca.

Without cutting anything locate the following structures in

1. The Female Urogenital System

Lift the ovary (39) *on one side; notice that it is attached to the body wall by*

81. the **mesovarium**, a thin sheet of peritoneum (32).
82. The **fat-body**, a mass of yellow finger-like processes, is attached to the cephalic end of the ovary.
83. The **kidneys (mesonephroi)**, a pair of red lobulated glands, lie underneath the ovary and are attached also to the fat-bodies. They are covered with peritoneum which obscures them in the preserved specimen, as they are extra-peritoneal.
84. The **ureter** or **mesonephric duct** may be seen at the lateral margin of each kidney by carefully removing the peritoneum. It leads to the cloaca.

85. The **adrenal gland**, a yellowish wavy streak upon the ventral side of the kidney, may also be seen. It is a ductless gland.
86. The two **oviducts** are highly convoluted white tubes. They open at the cephalic end by the
87. **ostia tubæ abdominales**, funnel-shaped apertures on the mesentery from the liver to the ventral body wall, just lateral to the root of the lung.
88. The oviduct is supported by a peritoneal membrane, the **mesotubarium.**

After completing the study of the visceral blood-vessels (page 14 ff.), slit open the cloaca and locate the openings of the excretory bladder, oviducts, and ureters.

Drawing 7. Make a drawing (x2) of the female urogenital system, showing the ovary on the left, the underlying organs on the right.

2. The Male Urogenital System

89. The **testis** is a yellow rounded body attached to the kidney (83) along its mesial edge by a membrane,
90. the **mesorchium.** At its cephalic end are fat bodies (82) similar to those in the female. Note also the adrenal gland (85) on the kidney, by tipping the testis towards the mesial line in order to expose the excretory organ.
91. Note the numerous, extremely small vessels passing along the mesorchium into the mesonephros. These are the **vasa efferentia.** Inside the mesonephros they unite with the mesonephric tubules of the kidney so that the spermatozoa may pass into the **mesonephric duct** (85), which, in the male, functions both as an ureter and a gonoduct.

92. At the points where the ducts enter the cloaca, they enlarge to form **seminal vesicles.**[1]

93. A rudimentary **oviduct**[2] lies alongside of each mesonephric duct.

Drawing 8. Draw (x2) the male urogenital system.

[1] Poorly developed in *R. pipiens* and *R. catesbiana.*
[2] Absent in *R. catesbiana.*

SECTION V. THE VASCULAR SYSTEM

The circulatory or blood-vascular system of the frog is a closed system, the blood being constantly confined within vessels. A heart, divided into two atria and one ventricle, pumps the blood through these vessels. From the ventricle the blood passes into one of three pairs of arches. The first, or carotid arches, carry blood to the head; the second, or systemic arches, carry blood to the rest of the body, uniting to form the dorsal aorta; the third, or pulmocutaneous arches, carry blood to the lungs and skin to be aërated. All the arteries terminate in tiny capillaries which unite to form veins. From the head and forelimbs the blood is returned by the precaval veins to the right atrium; from the viscera to the hepatic portal vein, terminating in the liver; from the hindlegs to the hepatic portal vein by the abdominal vein, or to the kidney by the renoportal veins; from the liver and the kidney to the right atrium by the postcaval vein; and from the lungs by the pulmonary veins to the left atrium.

You have already identified the cœliaco-mesenteric artery from the dorsal aorta to the viscera, and the hepatoportal veins (Drawings 5 and 6).

1. The Heart

It is desirable at this point to make use of a fresh specimen, preferably injected. Open along the mid-ventral line as before. Remove the sternum (235) *so as to expose the pericardial cavity* (29) *completely. Raising the pericar-*

dium, dissect it clear from the heart on the ventral side. Identify the following parts of the heart:

94. The **ventricle**, a thick-walled muscular sac, forms the apex of the triangular heart at its caudal end.
95. The **bulbus cordis** on the ventral surface of the heart arises from the ventricle on the right. It is separated by a slight groove from the
96. **truncus arteriosus**, a thinner walled tube assuming the form of a Y at the cephalic end of the heart. The two limbs of this Y lead to the aortic arches (p. 26).
97. Beneath the bulbus and truncus lie the **atria, right and left**, which are thin walled, distensible sacs.

Tip the heart forward and identify on the dorsal surface,

98. the **dorsal mesocardium**, a membrane which is suspended. *Cut this to see*
99. the **sinus venosus** which receives the blood from
100. the two large **precaval veins** from the cephalic side, and
101. the **postcaval vein** from the caudal end.
102. The **pulmonary vein**, which brings blood from the lungs, may be seen lying beside the left precaval vein. It enters the left auricle.

Drawing 9. Sketch the heart with its vessels (x2) from both dorsal and ventral aspects.

(The following dissection of the heart should be omitted if *R. catesbiana* is not available.)

Cut across the limbs of the truncus and the caval veins and remove the heart, identifying again the structures you have previously identified.
To trace the course of the blood through the heart, place it in a dish of water, dorsal side up. Carefully remove the

dorsal wall of the sinus and wash out the blood with the blowpipe. Find

103. the **sinu-auricular aperture**, leading from the sinus venosus (99) into the right auricle.

Place the heart ventral side up and remove the ventral wall of the auricles, taking care not to injure the truncus. Find

104. the **inter-auricular septum**, separating the two auricles (atria). Note the sinu-auricular aperture in the right auricle near the septum and
105. the **aperture of the pulmonary vein**, near the septum of the left auricle.

Again place the heart dorsal side up and remove the dorsal surface of the ventricle, to find

106. the **auriculo-ventricular aperture**, divided by the inter-auricular septum, at the cephalic end towards the ventral side, and
107. the **ventriculo-bulbar aperture**, leading into the bulbus, also at the cephalic end, but towards the dorsal side and to the right. Note
108. the ***semilunar valves*** by which this aperture is guarded. How many are there?

Slit open the bulbus and truncus and note

109. in the bulbus the **spiral valve**, dividing it longitudinally.
110. In the limbs of the truncus, observe the **septa** which divide them into three compartments, leading to the three aortic arches on each side.

2. The Veins

Returning to the injected frog, identify the cut end of the postcaval vein (101) *and follow this caudad, noting its tributaries as follows:*

111. There are three **hepatic veins** from the liver. These return the blood received from the hepato-portal veins (Drawing 6).
112. There are four or five **renal veins** from each kidney, which return the blood received from the reno-portal veins (115).
113. The **adipose veins** join the first pair of renal veins, which carry blood from the fat bodies.
114. The **ovarian** or **spermatic veins** convey blood from the ovary or testis respectively, and either join the renal veins or establish direct connections with the postcaval vein (101).
115. Note on the dorsolateral margin of each kidney the **reno-portal vein.** This receives branches from the body wall and the oviduct. Trace it back and note that it results from the union of
116. the **sciatic vein** from the dorsal surface of the thigh, and
117. the **external iliac vein** from its ventral surface. Trace back the external iliac until you find its juncture with the pelvic vein (78) running to the abdominal vein. The external iliac and pelvic are
118. branches of the **femoral vein,** the largest vessel returning blood from the hindleg. *If time permits trace back the sciatic and femoral veins and study the distribution of their tributaries in the leg.*

Return to the cut ends of the precaval vein. Trace forward the right precaval vein and note the following tributaries, by whose juncture it is formed (119, 123, 125).

119. The **external jugular vein**, returning blood from the head, is formed by the union of
120. the **lingual vein** from the tongue and floor of the mouth and

121. the **mandibular vein** from the lower jaw.
122. The second tributary of the precava is the **innominate vein** formed by the union of
123. the **internal jugular vein**, from the dorsal portion of the head, and
124. the **subscapular vein** bringing blood from the dorsal surface of the shoulder girdle and arm.
125. The third tributary of the precava is the **subclavian vein** which is formed by the union of
126. the **cutaneous vein** which returns the blood distributed by the cutaneous artery, and
127. the **brachial vein** which supplies the ventral portion of the arm. *If time permits, trace out the distribution of the subscapular and brachial veins.*

In the course of your dissection of the precaval vein, you will note three small bodies which are endocrine (ductless) glands.

128. Lying next to the mesial ventral surface of the external jugular is a small reddish brown oval body, the **pseudothyreoid (parathyreoid) gland.**
129. Somewhat dorsal and cephalad of this, the larger **thyreoid gland** may be seen.
130. The **thymus gland** may be found by tracing the internal jugular to the caudal margin of the tympanic membrane.

If these bodies have been destroyed they may be observed later during the dissection of the muscles.

Drawing 10. Make a sketch of the veins just dissected.

3. The Arteries

Trace the arteries on the right side of the heart, commencing with the cut end of the truncus arteriosus. This divides

*into three aortic arches (**131, 135, 138**). (See also p. 22.)*

131. The most anterior of the aortic arches is the **common carotid** which soon divides to form
132. the **external carotid (lingual) artery**, running to the lower jaw and ventral structures of the head, and
133. the **internal carotid artery**, a larger branch, running to the dorsal side of the head.
134. Note, at the juncture of these arteries, an oval dilation, the **carotid gland.**
135. The most posterior of the aortic arches is the **pulmocutaneous**, which divides into
136. a **pulmonary artery** to the lung and
137. a great **cutaneous artery** to the skin.
138. The middle arch is the **systemic**, which bends dorsally around the pharynx to unite with its mate from the opposite to form
139. the **dorsal aorta**, lying just ventral to the vertebral column. From the systemic arches spring four branches,
140. the **laryngeal artery**, to the larynx,
141. the **occipito-vertebral artery** to the skull and vertebral column,
142. The **œsophageal artery**, to the œsophagus, and
143. by far the largest, the **subclavian artery**. This vessel extends laterally and supplies the arm. *If time permits, trace its branches.*

Caudad of the union of the systemic aortic arches by which the dorsal aorta is formed, note the departure of the cœliaco-mesenteric artery (61) *and behind this, in sequence:*

144. four to six **urogenital arteries** supplying the kidney, fat body, ovary or testis,

145. one to four **lumbar arteries** to the body wall,
146. the **posterior mesenteric artery** to the rectum.
147. At its posterior end the dorsal aorta divides into two **iliac arteries.** Each gives off
148. a **hypogastric artery** to the urinary bladder and body wall, and
149. a **femoral artery** to the thigh, and then continues down the leg as
150. the **sciatic artery.** *Trace its branches if time permits.*

Drawing 11. Sketch the arteries you have identified.

Drawings 12 and 13. Assemble the sketches you have made of the arteries, veins, and heart (Drawings 5, 6, 9, 10, and 11). Prepare from these two diagrams, one to show the arterial circulation, the other to show the venous circulation.

SECTION VI. THE NERVOUS SYSTEM AND SENSE ORGANS

Anatomically, the nervous system is divided into three subsystems: 1, the peripheral system, a network of nerves conveying impulses from the sense organs, and to the muscles and glands; 2, the cerebro-spinal (central) system, a central co-ordinating tube; and 3, the autonomic (sympathetic) system supplying the viscera and associated with the peripheral system. The sense organs are the nose, eyes, and ears, together with scattered sensory elements in the skin and mouth.

Place the frog on the dissecting board, ventral side up, and carefully remove the urogenital system, taking care not to injure the dorsal aorta. Note that the kidneys are retro-peritoneal, and therefore outside of the cœlome. Identify the following structures.

151. The **periganglionic glands (calciferous bodies)**, are silvery white lymph glands surrounding the ganglia of the ten pairs of **spinal nerves**, which leave the vertebral column between the vertebræ.

152. The **first spinal (hypoglossal) nerve** is a very small nerve, leaving the spinal cord between the first and second vertebræ, and running cephalad and ventrad to the tongue. One or two fibres bend caudad to join

153. the **second spinal (brachial) nerve**, which is very large, and leaves the vertebral column between the second and third vertebræ and runs to the arm. It is joined by

154. the **third spinal nerve** which curves cephalad to meet the second and forms the **brachial plexus.**

155. The **fourth spinal nerve** runs caudad at an angle of forty-five degrees to the vertebral column and supplies the body wall.

156. The **fifth spinal nerve** is parallel to the fourth.

157. The **sixth spinal nerve** is similar to the fourth and fifth.

158. The **seventh spinal nerve** runs caudad, parallel to the vertebral column, and joins the eighth to take part in the formation of the **lumbro-sacral (sciatic) plexus.** Shortly before the point of junction, it gives off the **ilio-hypogastric** nerve to the body wall of the abdomen.

159. **The eighth** [1] **spinal nerve** is parallel to the seventh but mesiad to it. After its junction with the seventh it unites with the ninth spinal nerve. At this point a **crural nerve** is given off to the thigh.

160. The **ninth spinal nerve,** parallel and mesiad to the the eighth, unites with the other elements of the sciatic plexus to give rise to the great **sciatic nerve,** the largest single nerve in the body.

161. The **tenth spinal (coccygeal) nerve** is very small. It emerges from small openings in the urostyle (231) and after receiving a branch from the ninth to form the **ischio-coccygeal plexus,** supplies the cloaca and bladder.

(If time permits, trace the brachial and sciatic nerves to the toes.)

162. The **sympathetic trunks** are longitudinal nerves of the autonomic system lying along the systemic arches and dorsal aorta. Each is connected by

[1] Largest element of the sciatic plexus of *R. catesbiana.*

163. rami **intercommunicantes** with the spinal nerves.
164. The **splanchnic nerve** runs along the cœliaco-mesenteric artery (**61**) and receives branches from the third, fourth, fifth, and sixth sympathetic ganglia, which unite with each other to form the **solar plexus**.

Drawing 14. Draw the ventral surface of the dorsal body wall, labeling all the structures you have just identified (x2).

Place the frog dorsal side uppermost. Make a transverse incision in the skin across the head just cephalad of the arms, strip the skin forward, and note:

165. The slender **columella** is in the aperture lying beneath the tympanum (6). Note its relation to the tympanum.
166. There is a **tympanic cavity** (middle ear).

*Carefully remove an eyeball, by cutting through the eyelids, the muscles of the eyeball, and the optic nerve (**193**). Cut the eye into two portions, by cutting across it with scissors to one side of the pupil in any except the sagittal (vertical longitudinal) plane. Place the larger portion in a dish of water and note the following structures.*

167. The transparent **cornea** at the external (exposed) surface above the iris, continues into
168. the inner opaque **sclerotic layer**.
169. The thin black pigmented **chorioid layer** extends outward under the cornea as the iris (**3**).
170. A thin, yellow-white layer at the proximal half of the eye is the sensory **retina**.
171. The **crystalline lens** is a large solid body which is transparent in a fresh specimen.

Drawing 15. Make a diagram of the eye on the basis of this dissection (x2).

Remove the muscles from the back of the head. Bend the head ventrally until a space is formed between the skull and the vertebral column. It exposes a dark membrane. Remove this with forceps and the white brain will be seen beneath it. Make two longitudinal cuts with scissors through the dorsal surface of the skull, taking great care to cut upward so as not to injure the brain. Slowly whittle away the skull roof until the brain is exposed. Do not attempt to remove it at this time. Identify the following parts from the dorsal side.

172. The **olfactory lobes (rhinencephalon)** are at the level of the caudal margins of the eyes. They give off the olfactory (first cranial) nerves (181) from their cephalic ends.
173. The **cerebral hemispheres (telencephalon)** are separated from the olfactory lobes by a shallow groove. They are separated from each other by a **sagittal fissure.**
174. The **diencephalon** lies caudal and a little ventral to the telencephalon. At its anterior end is a small cavity, covered, in life, by the **anterior chorioid plexus** which may not appear in a preserved specimen. Behind this, in exceptionally good dissections, may be seen
175. a small, dark, vascular body, the **paraphysis,** and
176. a thin, small tube, the **epiphysis (5).**
177. Caudal to the diencephalon and rising dorsally, are the **optic lobes (mesencephalon).**
178. Caudal to this and at a more ventral level, is a small transverse fold, the **cerebellum (metencephalon).**
179. The most caudal division of the brain is the **medulla oblongata (myelencephalon),** which narrows at its caudal end to join the spinal cord (180). Its roof

is thin and covered by the **posterior chorioid plexus** which usually comes off with the overlying bone, to expose the fourth ventricle (201).

180. The **spinal cord** should be traced back as far as the first spinal nerve (152).

The cranial nerves of the frog are small and the dissection of them is difficult. Identify the following nerves from the dorsal aspect.

181. The **olfactory nerve (I)** arises from the olfactory lobe and runs to the nose.
182. The **trochlear nerve (IV)**, very small, arises from the brain between the optic lobes and the cerebellum. (It supplies one of the eye muscles.)
183. The **trigeminal nerve (V)** arises from the medulla at its cephalo-lateral angle and passes into the **proötic ganglion**, where it is joined by
184. the **facial nerve (VII)**.

From the proötic ganglion trace forward five branches.

185. The caudal branch is the **hyomandibular ramus of the VIIth.**
186. Anterior to this the **maxillo-mandibular ramus of the Vth** arises.
187. Next the **temporal ramus of the Vth** is given off.
188. Ventral to these is the **ophthalmic ramus of the Vth.**
189. Ventral to the hyomandibular ramus, the **palatine ramus of the VIIth** takes its origin.
190. The **auditory nerve (VIII)** leaves the brain close behind the VIIth and passes directly to the ear.
191. The **glossopharyngeal nerve (IX)** arises from the sides of the medulla by four roots which it possesses in common with the tenth cranial nerve, with which it is associated.

192. The **vagus nerve (X)** is associated with the IXth, along with which it leaves the skull. Outside the skull they expand to form the **jugular ganglion** and from this they emerge as separate nerves.

Drawing 16. Draw the brain and cranial nerves from the dorsal aspect (x2).

Remove the brain by cutting through the cranial nerves, commencing with the olfactory, and place the brain ventral side up. Identify the following structures.

193. The **optic nerves (II)** unite in the **optic chiasma.**
194. The **infundibulum** is a bilobed projection, often removed in dissecting, caudal to the chiasma.
195. Attached to the distal end of the infundibulum, and caudal to it, is the somewhat trilobed **pituitary gland**, another endocrine organ.
196. The **oculo-motor nerves (III)** arise dorsal to the lobes of the infundibulum and supply certain muscles of the eyeball.
197. Close to the mid-line of the medulla are the small **abducens nerves (VI),** each of which after sending a twig to the proötic ganglion proceeds to one of the muscles of the eyeball.

Drawing 17. Sketch the ventral surface of the brain indicating the nerves that arise from it (x2).

With a sharp scalpel remove the roof of the brain, noting the following cavities.

198. In the telencephalon, the **lateral ventricles** connected by the **foramen of Monro** make up the **telocœle.** These are the first and second ventricles.
199. The foramen communicates with the **third ventricle** or **diacœle** in the diencephalon.

200. In the mesencephalon, the **mesocœle** consists of the **cavities** of the optic lobes and the **iter** or **aqueduct** into which they open. This passage connects the diacœle with
201. the **fourth ventricle** or **myelocœle**, already noted (179). This cavity narrows into
202. the **central canal** of the spinal cord.

Remove the roof of the auditory capsule (225) *and note:*

203. the **membranous labyrinth** of the inner ear. Unusually favorable dissections reveal
204. the dorsal **utriculus** with its
205. three **semicircular canals**, each with its **ampulla**,
206. and the ventral **sacculus**, with
207. a delicate tube, the **ductus endolymphaticus**, running dorsad from the cephalic margin.

SECTION VII. THE SKELETON

If a prepared skeleton is not available, skin a frog and remove the viscera and as much of the musculature as possible. Boil for five minutes (repeated if necessary) in soap solution (see Appendix), and pick away the remainder of the soft parts.

The skeleton of the frog is entirely an endoskeleton. It is composed of the axial skeleton, including the skull, vertebræ and sternum; and the appendicular skeleton, consisting of the pectoral and pelvic girdles and the bones of the limbs.

The skull consists of certain derm-bones which form the dermocranium (D), axial elements which form the neurocranium (N), and derivatives of the gill arches (visceral skeleton) which form the splanchnocranium (S).

In the skull identify the following bones from the dorsal aspect, commencing at the cephalic end.

208. The **premaxillaries (D)** are the most anterior bones bearing teeth.
209. The **maxillaries (D)** also bear teeth and form the greater part of the upper jaw.
210. Articulating with the premaxillaries at their caudal ends are the **nasals (D)**, which send long processes laterad to join the maxillaries.
211. Caudal to the nasals, and partially overlaid by them, is the **sphenethmoid (N)**.
212. This is overlaid caudad by the long **fronto-parietals (D)**.

213. Caudal and ventral to these are the **occipitals (N)**, bearing the **condyles** by which the skull articulates with the atlas (228) of the vertebral column. Between these, on the posterior face of the skull, is the **foramen magnum**, the apèrture by which the spinal cord leaves the skull.
214. Laterad to the occipitals and articulating with the fronto-parietals are the **proötics (N)** which cover the inner ear. Note the opening, **fenestra ovalis**, covered in life by a cartilage, with which the columella (165) articulates.
215. Laterad to the proötics are the large T-shaped **squamosals (tympanics) (D)**.
216. At the caudal lateral margins of the squamosals are the **quadrato-jugals (D)**, which articulate with the maxillaries. They form the suspension for the lower jaw.

Drawing 18. Draw the right half of the skull from the dorsal aspect (x4).

Place the skull ventral side up. Examine the lower jaw and find the following bones.

217. At the cephalic end, corresponding to the premaxillaries, are the **mento-meckelians (predentales) (D)**.
218. The long **dentales (D)** are just caudal to them. Note the arrangement of the teeth.
219. The **angulare-splenials (D)** articulate with the quadrato-jugals (216) by means of the **quadrate cartilage (S)**. These derm-bones cover the **Meckelian cartilages (S)** of the lower jaw. Both cartilages arise from the first (mandibular) visceral arch of the embryo.

Remove the lower jaw and identify the following bones on the ventral surface of the skull:

220. Articulating with the premaxillaries are the tooth-bearing **vomers** (D).
221. Caudal to these are the thin splint-like **palatines** (D).
222. The longitudinal **parasphenoid** (D) extends caudad.
223. The triradiate **pterygoids** (S) articulate with the maxillaries and nasals cephalad, the parasphenoid mesiad, and the quadrato-jugal at the angle of the jaw.

In a wet specimen note also the following cartilages from which the derm-bones have been removed:

224. The **nasal capsule** (N) surrounds the nose.
225. The **auditory capsule** (N) surrounds the ear.
225. The **subocular arch** (N) connects the nasal and auditory capsules with the quadrate cartilage (219).
227. The **hyoid cartilage** (S) supports the tongue.

Drawing 19. Draw the left half of the skull from the ventral aspect (x2).

Examine the vertebral column, consisting of ten bones.

228. The **atlas** supports the skull. This is the only cervical vertebra the frogs possess.
229. There are seven **abdominal vertebræ.** Note the dorsal **neural spines**, the **cephalic processes** (prezygapophyses), **caudal processes** (postzygapophyses), and **lateral transverse processes** (diapophyses). The latter articulate with short cartilages sometimes considered ribs.[1]
230. The **sacral vertebra** has, united to it, the ilium of the pelvis (242).

[1] See Kingsley, *The Vertebrate Skeleton*, p. 41.

231. The **urostyle (coccyx)** represents the fused **caudal vertebræ**. Note the small apertures by which the tenth spinal nerve emerges.

Examine the breast bone from the ventral aspect, identifying the following bones:

232. At the cephalic end is the **episternum**, with a cartilaginous plate projecting from it cephalad. At the caudal end it articulates with
233. two derm-bones (not part of the sternum proper), the **clavicles**, which run laterad to the scapulæ (236).
234. Caudad of the clavicles are two stout transverse bones, the **coracoids**, elements of the pectoral girdle.
235. The most caudal structure is the **sternum**, terminating in a cartilaginous plate.

Examine the pectoral appendage, shoulder and arm, and identify the following bones. The girdle consists of the coracoid (234), and a cartilaginous procoracoid partially covered by the clavicle (233), and two other elements (236 and 237).

236. The **scapula** is a lateral element bearing on its lateral margin a notch, the **glenoid fossa**, the remainder of which is formed by the coracoid.
237. The dorsal bone is the **suprascapula**.

The free appendage contains twenty-three bones; identify the following:

238. The proximal bone is the **humerus**, fitting into the glenoid fossa.
239. Distal to this is the **radio-ulna**, resulting from the fusion of the radius and ulna of the embryo.
240. This is followed by the **carpus** or wrist containing

six bones, the proximal **ulnare**, **centrale** and **radiale**, and the distal **carpales**.

241. The distal group of bones is the **manus** or hand, containing the five **metacarpales** and the **phalanges**, two for each of the inner digits, three for the outer digits, fifteen bones in all.

Examine the pelvic appendage (pelvis and leg) and identify the following bones:

The girdle consists of:

242. the long, sword-shaped **ilium**, articulating at its cephalic end with the ninth vertebra,
243. the **ischium**, caudal to the illium, and
244. the **pubis**, ventral to the two others. The **acetabulum**, or cup into which fits the head of the femur (245), is formed by the union of these three bones.

The free appendage contains twenty-eight bones.

245. The proximal bone is the **femur**, its head fitting into the acetabulum.
246. Distal to this is the **tibio-fibula**, resulting from the fusion of the embryonic tibia and fibula.
247. This is followed by the **tarsus** or ankle containing five bones, the **tibiale**, **fibulare**, **centrale** (at the base of the prehallux (10), and two **tarsales**.
248. The distal group of bones is the **pes** or foot, containing two small bones of the prehallux,[1] five **metatarsales**, and the **phalanges**, two in the first and second digits, three in the third and fourth digits, and four in the fourth digit, twenty-one bones in all.

[1] The prehallux may have from one to three elements.

SECTION VIII. THE MUSCULATURE

For the study of the musculature in detail a fresh specimen of *R. catesbiana* is desirable. If this is not feasible, the dissection of the muscles of the leg (pp. 44-6) at least should be completed, using the material already at hand. The muscular system includes not only the muscles attached to the skeleton but also the muscles of the viscera, skin, and sense organs. The skeletal muscles alone will be considered in this guide, and only the more prominent of these. The advanced student is referred to Gaupp, where over one hundred and fifty separate skeletal muscles are described. In the description of the muscles, the term *origin* refers to the relatively immovable attachment of the muscle, the term *insertion* to the part moved. In dissecting the muscles, they should be spread apart with the handle of the scalpel. When the directions call for the cutting of a muscle, this should be done by lifting it carefully and making an incision through the middle (*belly*) of the muscle from below.

Skin a frog, exercising great care not to tear any of the underlying structures. Place it on the dissecting board and identify the following muscles, commencing at the cephalic end. Sketch as you dissect, showing the superficial muscles at the right of the mid-line of your drawing, and the deeper muscles at the left. Do not attempt to verify the insertions and origins until you have identified the adjacent muscles.

249. **Temporalis.** In front of the tympanum. Origin from proötic and occipital: insertion on angulare.

250. **Depressor mandibulæ.** Behind tympanum. Ori-

gin from **dorsal fascia** (connective tissue along midline of the back): insertion on lower jaw.

251. **Dorsalis scapulæ.** Covering suprascapula. Origin from suprascapula: insertion on humerus.

252. **Latissimus dorsi.** Caudal and dorsal to dorsalis scapulæ. Origin from dorsal fascia: insertion on humerus.

253. **Longissimus dorsi.** A longitudinal muscle, caudal and ventral to the latissimus. Origin from urostyle: insertion on occipitals. It is marked by a number of transverse white lines, the **septa tendinea.**

254. **Coccygeo-sacralis.** At the lateral margin of the caudal end of the longissimus. Origin from urostyle: insertion on sacral vertebra.

255. **Coccygeo-iliacus.** Caudal and lateral to coccygeo-sacralis by which it is covered cephalad. Origin from urostyle: insertion on ileum.

256. **Obliquus externus.** The broad lateral muscle of the body wall. Origin from dorsal fascia: insertion on ventral **aponeurosis** (corresponding to dorsal fascia on ventral side). One portion (**pars scapularis**) at the cephalic end connects the suprascapula with the sternum.

Cut through the obliquus externus and note ventral to it:

257. **Transversus**, a similar sheet, but with its fibers at a different angle. Origin from ileum, fascia dorsalis and the fourth vertebra: insertion on ventral aponeurosis.

Carefully pull the depressor mandibulæ cephalad and lift suprascapula exposing the following muscles:

258. **Rhomboideus anterior**, at the cephalo-mesial border. Origin from dorsal fascia and fronto-parietals: insertion on ventral surface of suprascapula.

259. **Levator scapulæ superior**, lateral to the rhomboideus anterior. Origin from the proötic, and squamosal: insertion on ventral surface of suprascapula.

260. **Levator scapulæ inferior**, lateral and ventral of the rhomboideus anterior. Origin from occipital: insertion on ventral surface of suprascapula.

261. **Cucularis**, at the lateral margin, a long thin muscle. Origin from proötic and occipital: insertion on cephalic margin of scapula. Note between the cucularis and the depressor mandibulæ the thymus gland (130).

Pull the latissimus dorsi caudad and lift suprascapula, exposing

262. **Rhomboideus posterior** at the mesial border. Origin from 3d and 4th vertebræ: insertion on suprascapula.

263. The **serrati**, three small muscles arising from the 3d and 4th vertebræ, two of which insert on the suprascapula, and the third on the scapula.

Drawing 20. Complete sketch of dorsal body musculature (x2).

Turn the frog ventral side uppermost and identify the following muscles, commencing a sketch as before:

264. The **submaxillaris**, uniting the halves of the lower jaw.

265. **Subhyoideus**, immediately caudad. Origin from the hyoid: insertion on lower jaw.

266. Extending caudad from beneath the submaxillaris, the **geniohyoideus** on each side of the mid-line. Origin from lower jaw: insertion on hyoid.

267. Between and dorsal to these, the **hyoglossus**. Origin from hyoid: insertion in tongue.

268. Laterad, the **deltoideus** passes to the arm. It has three portions originating from the sternum, clavicle and scapula respectively and inserted on the humerus.

269. Overlying this and caudad, the great **pectoralis** extends from the sternum to the humerus. It has three origins, the epicoracoid, sternum, and the sheath of the rectus abdominis (270).

270. Caudad to the pectoralis, the **rectus abdominis** extends longitudinally on each side of the mid-line from the pubis to the sternum. Note the white transverse tendonous inscriptions. At the sides the obliquus externus (256) may be seen.

Pull the deltoideus cephalad and the pectoralis caudad, exposing between them,

271. the **coraco-radialis**, with its broad origin along the episternum and insertion on the radial portion of the radio-ulna.

Cut through the deltoideus and the pectoralis and identify:

272. the **coraco-brachiales**, two slender transverse muscles. Origin from coracoid: insertion on humerus.

273. **Anconeus (triceps)**. This great muscle of the upper arm has one head originating from the scapula and three from the humerus. The insertion is on the ulnar side of the radio-ulna. (The dissection of the muscles of the lower arm and hand are omitted in this manual.)

Cut through the submaxillaries in the median line, exposing

274. the **submentalis**, uniting the tips of the lower jaws.

Retract the sternum, exposing

275. the **sternohyoideus** with origin from the sternum and insertion on the hyoid.
276. The **omohyoideus** with its origin on the scapula and insertion on the hyoid. In the angle formed by these muscles as they converge towards the hyoid, note the parathyreoids (128).

Cut through the sternohyoideus and observe:

277. the **petrohyoidei**, four small muscles from the auditory capsule (225) to hyoid. Note in the notches of the hyoid, close to the origin of the hyoglossals, the thyreoid glands (129).

Open the jaws widely, remove the skin from the mouth and identify the temporalis (249) *and the following:*

278. **pterygoideus.** Cephalad and mesiad of the temporalis. Origin from the fronto-parietal and proötic: insertion on lower jaw.
279. The **masseters**, two small muscles of which one runs from the cephalic border of the tympanum to the angulare, concealing the other which connects the quadrato-jugal with the angulare.

Drawing 21. Complete drawing of ventral body musculature (x2).

The Muscles of the Hind Leg

Place the frog ventral side up and identify the following muscles, making a sketch as you proceed.
In the thigh, commencing at the lateral margin:

280. **Triceps femoris.** This great muscle has three origins; the **caput anticum** from the cephalic margin of the acetabulum (244), **caput medium** from the ilium, and **caput posticum** from the same bone at the caudal end: insertion on tibio-fibula.

281. **Sartorius**, a flat strip of muscle, extending straight down the thigh. Origin from ilium: insertion on tibio-fibulare.
282. **Adductor magnus**, large and thick, mesial of the sartorius, beneath which it passes distally. Origin from pubis and ischium: insertion on femur.
283. **Gracilis (rectus internus) major**, a large muscle at the mesial border of the thigh. Origin from ischium: insertion on tibio-fibula.
284. **Gracilis (rectus internus) minor**, a small thin muscle, forming the mesial margin of the thigh. Origin from ischium: insertion with that of the gracilis major.

In the shank commencing at the lateral margin:

285. The **tibialis anticus longus**. Origin from femur: insertion on tibiale and fibulare.
286. **Extensor cruris**, a slender muscle lying close to the tibio-fibula. Origin from femur: insertion on tibio-fibula.
287. **Tibialis anticus brevis**, very short. Origin from tibio-fibula: insertion on tibiale.
288. **Tibialis posticus**, a long thin muscle lying close to the mesial side of the tibio-fibulare from which it takes its origin: insertion on tibiale.
289. **Plantar longus (gastrocnemius)**, the largest leg muscle, at the mesial margin. Origin from two heads, one from a tendon connecting femur and tibio-fibula, the other from tendon of triceps: insertion in a broad tendon (aponeurosis) on the sole of the foot.

Drawing 22. Draw the ventral musculature of the hind leg (x2).

From the dorsal aspect, identify the following muscles. Sketch.

In the thigh, commencing at the lateral margin:

290. At the proximal end the small **iliacus externus.** Origin from ilium: insertion on femur. At its distal end it is obscured by the caput posticum of the triceps (280).

291. From the urostyle another small muscle, the **pyriformis**, extends to the head of the femur.

292. Mesial of the caput posticum, the slender **iliofibularis.** Origin from ilium: insertion on tibio-fibula.

293. The large muscle at the mesial margin is the **semimembranosus.** Origin from ischium: insertion on tibio-fibula.

In the shank, in addition to the plantar longus (289) *and tibialis anticus longus* (285), *note*

294. the **peroneus**, between the two just noted. Origin from femur: insertion on fibia and fibulare.

Cut through the sartorius (281) *and identify:*

295. The **adductor longus**, a narrow muscle covered by the sartorius. Origin from the ilium: insertion with that of adductor magnus (282).

296. The **iliacus internus** may now be identified. It extends from the ventral border of the ilium to the femur.

Cut through the gracilis major (283) *and identify*

297. the **semitendinosus**, a delicate muscle with two heads, covered by the gracilis major. Origin from ischium: insertion on tibio-fibulare.

The deeper muscles of the thigh, six in number, as well as the muscles of the foot, are omitted.

Drawing 23. Draw the dorsal musculature of the hind leg (x2).

SECTION IX. HISTOLOGY

The slides loaned you have been prepared by preserving the tissues, cutting them in thin slices (sections) (except the blood, which has been smeared on the slide) dyeing them in contrasting colors, and mounting them in a transparent resin under the coverslip. By careful study you can identify on them examples of the principal tissues of the vertebrate body.

1. Blood

In the slide given you observe

298. the **erythrocytes** (red blood corpuscles). These are elliptical cells with deeply staining oval nuclei.

299. The **leucocytes** (white blood corpuscles). Much fewer in number, these may be recognized by their irregular shape, large granular and often irregularly shaped nucleus and the occasional presence of granules in the cytoplasm.

300. The **blood-plasma** is represented by the faintly staining background between the cells. Some slides may show a few twisted elongated cells with small nuclei and flagella. These are parasitic protozoa, *Trypanosoma ranarum.*

Drawing 24. Draw examples of erythrocytes and leucocytes highly magnified.

2. Cartilage (Hyaline)

In the slide provided you, identify

301. the **cartilage cells**, small, ovoid and often associated in groups of two, four, etc. Study under high power.

302. The **matrix**, a lightly stained mass (transparent in life) with spaces, **lacunæ**, in which the cells appear.
303. The **perichondrium**, a layer of connective tissue with small blood vessels, covering the cartilage.

Drawing 25. Draw a section showing all structures mentioned above.

3. Bone (Transverse Section of Femur, Decalcified)

In the slide provided, note the following:

304. The **periosteum**, a layer of connective tissue, resembling the perichondrium (303).
305. The outer zone of **compact bone**. Study with high power and observe
306 the irregularly star-shaped **bone cells.**
307. The **matrix** with its concentric layers, the **laminæ**, the **lacunæ** surrounding the cells, the widely branching delicate canals leading from them, the **canaliculi.** Sometimes the cells are grouped around cavities containing blood vessels and nerves. These cavities are called **Haversian canals.**
308. The **bone marrow**, forming the center of the femur. It is essentially an adipose connective tissue with many blood corpuscles. Examine under high power.

Drawing 26. Draw in outline the entire section. Fill in the details in a narrow sector only.

4. Striated Muscle. (Longitudinal Section)

In the slide loaned you, identify:

309. The **muscle cells** or fibers. They are grouped in bundles or **fasciculi.** Study under high power and note in each fiber several nuclei, longitudinal striations (**sarcostyles**), transverse striations (dividing

the fiber into **sarcomeres**), and the delicate cell membrane (**sarcolemma**).

310. The **connective tissue cells**. Inside the fasciculus they are called **endomysium**, around the fasciculi they are known as **perimysium**, while outside the muscle they form the **epimysium**.

Drawing 27. Sketch a section of the muscle and a detail of one fiber.

5. Nerve. (Cross-section of Sciatic Nerve)

In the sections of the nerve, observe:

311. The individual **nerve fibers**. Unlike the muscle fibers each is composed of more than a single cell. In the individual fiber observe under high power
312. the **axis cylinder**. Under the highest powers fine dots, cross-sections of fibrils, appear. The fiber is surrounded by a delicate **exolemma**. The nuclei are in the spinal cord or ganglia.
313. The **medullary sheath** surrounds the axis cylinder.
314. The sheath is in turn surrounded by a delicate membrane, the **neurolemma**. The nuclei of the neurolemma are small and compact.
315. Around the nerve is a layer of connective tissue, the **perineurium**.

Drawing 28. Draw a sector of the nerve under high power.

6. The Skin

In the section of the skin, identify the following structures.

316. The **epidermis**, stratified epithelium. It consists of two layers,
317. the **stratum corneum**, squamous epithelium, at the outer surface, and

318. the **stratum germinativum (Malpighii)**, columnar epithelium, below the corneum.

319. The **dermis** (**corium**) underlies the epidermis and is connective tissue. Like the epidermis it is divided into two layers,

320. the **stratum spongiosum**, towards the exterior, which is a loose layer containing the **dermal glands** (323), and the pigment cells (**chromatophores**), and

321. the **stratum compactum** beneath containing both white and yellow fibers, some smooth muscle cells, blood vessels and nerves.

322. Beneath the dermis is the **sub-cutaneous** layer of connective tissue which connects the skin with the muscles of the body wall. This is a loose connective tissue, divided into two layers by the lymph spaces.

323. The glands are of two types, the more frequent and smaller **mucous glands** and the relatively rarer and larger **poison glands**. The glands are composed of a layer of epithelial secretory cells, surrounded by a layer of smooth muscle cells, which in turn is covered with fibrous connective tissue. Each gland communicates with the exterior by a **duct** whose aperture is called a **stoma.**

Drawing 29. Draw a section of skin to show as many of these structures as possible.

7. The Intestine (Transverse Section of Ileum)

In the section provided, locate the following structures:

324. The interior of the tube is lined by the **mucosa**, which is secretory columnar epithelium. Under high power distinguish the **goblet cells** which have an oval vacuole filled with mucus at the inner end.

325. Exterior to the mucosa lies the **submucosa,** an ex-

cellent example of areolar connective tissue, with fibers, blood vessels and lymph spaces.

326. Outside the submucosa is a thick layer of **circular smooth muscles** and

327. a thin layer of **longitudinal smooth muscles.**

328. The exterior layer is a thin covering of fibrous connective tissue and of squamous epithelium, the **peritoneum.** Many capillaries may be seen.

Drawing 30. Draw an outline of the intestine, filling in the details in one sector only.

SECTION X. EMBRYOLOGY

Within the limits of this manual, it is possible to give directions for a rapid survey of the principal external features only of the development of the frog. This survey can be performed most advantageously in the spring when living material is available. But as this is not always feasible, the directions are based on preserved material. This will be given you in watch-glasses to be studied with the aid of a hand-lens. Be careful in handling the material. When you desire to change the position of an egg or embryo do so by using a gentle stream of water from a pipette.

1. Cleavage and Germ Layer Formation

Examine the following stages.

329. The **fertilized egg** before cleavage (one-cell stage). Note particularly: the **egg-envelope** (jelly), the **apical (animal) hemisphere** with its black **pigment**, the **white abapical (vegetative) hemisphere.** It is sometimes possible with fresh material to see the small **polar bodies** at the apical pole, and the **gray crescent** between the apical and abapical hemispheres. (Demonstration of spermatozoa may be shown at this time.)

330. The **first cleavage** (two-cell stage). Note the first cleavage furrow. What relationship has it to the apical pole (and the gray crescent, if present)?

331. The **second cleavage** (four-cell stage). Note the first two cleavage furrows. What is their relation-

ship to the structures already noted and to each other? Note that the furrows do not form a right angle at the apical pole; the small cross furrow is called the **polar furrow**. How is it produced?

332. The **third cleavage** (eight-cell stage). Note the third, meridional, cleavage furrow. What relationship has it to the first two and to the hemispheres? Which of the cells (**blastomeres**) are the larger?

333. The **fourth cleavage** (twelve- and sixteen-cell stages). Note that the fourth cleavage is produced by two furrows. What is their relationship to each other, and to the other cleavage furrows? How can you account for the existence of the twelve-cell stage?

334. The **fifth cleavage** (twenty-four and thirty-two cell stages). The fifth cleavage is also produced by two cleavage furrows. How does their relationship to each other differ from that of the two furrows in the fourth cleavage? How can you account for the existence of a twenty-four cell stage? Are the blastomeres equal in size and regular in arrangement, or not?

335. The **blastula**. Note the small size of the cells. *Hemisect* through the apical pole and note the cavity (**blastocœle**). Examine under binocular microscope if possible. If blastulae are not available, this exercise can be performed on the fifth cleavage stage (334). The cavity is then called the **segmentation cavity**.

336. A late **gastrula** (yolk-plug stage). Note the small white protuberant **yolk-plug**, which occupies the potential blastopore. All the rest of the surface is composed of minute pigmented cells, the **ectoderm**. *Hemisect* the gastrula through the yolk-plug and

observe a small excentric cavity, the **gastrocœle**. It is lined with minute white cells, the **endoderm**. The third germ layer, the **mesoderm**, may be shown in demonstration by prepared sections.

Drawing 31. Draw these stages including the hemisections (9) in such a way as to show the greatest number of structures on each. Make each drawing about two inches in diameter.

2. Early Embryos and Larvæ

Examine the following stages:

337. An **early embryo (neural groove stage)**. Note on the dorsal aspect the **neural folds**, meeting at the cephalic end, and approximating each other at the caudal end. Between the neural folds is the broad **neural plate**. The **blastopore** is a narrow slit between the caudal ends of the neural folds. What has become of the yolk-plug?

338. An **early embryo (neural tube stage, 3 mm. long)**. The neural folds have now united to form a **neural tube**. Note in the head region, the **stomodæum** (the future mouth), a narrow slit on the ventral surface, enclosed by ridges which will become the jaws. The beginnings (primordia) of the **oral suckers** may be seen ventral to the stomodæum. On each side of the head is a bulge caused by the developing **eye**. Behind this is a swelling known as the **gill plate**, with five furrows, the **visceral (gill) grooves**. Behind the last is a swelling caused by the **pronephros**. On the dorsal side of the body are slight furrows indicating the presence of **somites**. A ventral bulge is the **yolk-sac**. There is a slight **tail bud**, beneath which is a small **vent**.

339. The **larva at hatching** [1] (6 mm.). Note the changes that have taken place since the 3 mm. stage in the structures mentioned above. On the third and fourth visceral (gill) arches, **external gills** have appeared. Dorsal and lateral to the margins of the stomodæum are two minute **olfactory pits** which are the beginnings of the nose.

Drawing 32. Draw these three stages one above the other, from the left side, magnified about twenty times.

3. The Dissection of the Tadpole (*Rana catesbiana*)

The bullfrog spends the first two years of its existence in the larval or tadpole stage. The large size of the tadpole offers an excellent opportunity for a dissection of the larval organs and a comparison with those of the adult.
Place the tadpole on its right side and observe:

340. the external characters. What changes have taken place since hatching? Find the ventral **mouth**, armed with horny **teeth**; the tiny **nostrils**, one about a quarter of an inch dorsal of each side of the mouth; the **eyes**; the **opercular aperture** half way down the trunk on the left side; the **tail**, with its **fin**; and at the base of the latter locate the tiny **hind limbs**. The **vent** is at the base of the fin on the right side.

Drawing 33. Make a drawing of the tadpole from the left side. Place the tadpole on its back and remove the body wall from the ventral surface, taking care not to injure the opercular aperture.

[1] The larva of *R. pipiens* under laboratory conditions varies in length from 3 to 6 mm. at the time of hatching.

341. Note the **heart** in its **pericardial cavity**. On either side find the **internal gills**, in the **opercular cavity**. Probe through the opercular aperture. Count the gills, and note the **gill (visceral) clefts** between them. Probe through the mouth. Deep in the caudo-lateral angles of the opercular cavity find the diminutive **forelimbs**. In the visceral cavity note the long coiled **intestine**. *Lay it to one side after cutting through the rectum at the caudal end and the dorsal mesentery by which it is attached to the body wall. Do not cut through the cephalic end.* At the cephalic end, note the **stomach** and slender yellow **pancreas**, the **liver** and **gall bladder**, and the **œsophagus**. Compare with the digestive system of the adult. Find the **lungs**. On the dorsal surface of the cœlome, note the conspicuous yellow **fat-bodies** attached to the **kidneys**, relatively larger than in the adult, and the rudimentary **gonads**.

Drawing 34. Draw (x2) from the ventral surface, showing the intestine displaced to your left, in order to expose the organs normally concealed by it.

Remove tail by a transverse incision behind the vent.

342. Examine cut surface and locate: **notochord**, **spinal cord**, **caudal artery**, and **muscles**.

Drawing 35. Draw (x4).

APPENDIX

The supplies for each student required to complete the directions in this manual are:

One injected frog (preferably doubly injected).
One frog, which need not be injected.
One mounted skeleton of frog. This is desirable but not indispensable.
Seven histological slides, including one each of the following: blood smear; section of hyaline cartilage; transverse section of decalcified femur; longitudinal section of striated muscle; transverse section of sciatic nerve; section of skin; transverse section of ileum.
One set, ten stages in the development of the frog.
One bullfrog tadpole.

All these may be readily obtained from any of the biological supply stores. The following suggestions may be of service to the teacher who prefers to prepare his own material.

The best time to collect the frogs is at night and during the breeding season, in the Spring, when they can easily be netted with the aid of a lantern or electric torch.

They may be kept in large tanks with from one to two inches of water at the bottom, which should be changed frequently. Overcrowding the tanks should be avoided. Frogs need not be fed during the winter months if kept in a cool place. If feeding is to be demonstrated, it should be remembered that the frog will take only moving food.

The best method of killing the frogs is by the fumes of ether. When the tissues are to be preserved for microscopic preparation, the preferable method is that of "pithing." Locate with the fingernail the point at which the skull joins the vertebral column, press the head down sharply and make a narrow, deep, transverse incision at the point located. This severs the spinal cord, and if done neatly, results in practically no loss of blood.

To preserve the frogs, use formaldehyde in 5% aqueous solution. The commercial formaldehyde is 40% formalin. Mix 5 parts of

this with 95 parts distilled water. The body cavity should be opened, a little to one side of the mid-line, to insure good preservation of the viscera.

Injected specimens may be prepared by injecting the arteries through the truncus arteriosus (single injection), the hepatoportal veins through the abdominal vein (double injection), and the remaining veins through the postcava (triple injection). The most useful mass is prepared with cornstarch as follows:

Cornstarch	4 parts by volume
Chloral hydrate, 2% aqueous sol.	4 "
Alcohol 95%	1 "
Color and glycerine (equal parts)	1 "

Mix and stir, strain through cheesecloth.

For colors use vermilion for arteries, insoluble Prussian blue for hepatoportal vein, chrome yellow for postcava.

Skeletons are prepared by boiling in the following soap solution:

Soap	75 grams
Potassium nitrate	12 "
Ammonia	150 cc.
Distilled water	2000 cc.

Heat the water before adding the other ingredients. Use diluted, one part of the mixture to three parts of water.

To decalcify the skull before dissecting the brain use the following mixture:

Alcohol, 95%	1 part by volume
Nitric acid, 10% sol.	1 "

For the preparation of the slides called for, it will be necessary to consult the works on microscopic technique mentioned in the Introduction. The blood smears may be stained with any of the regular blood stains, although hæmatoxylin and eosin give preparations quite adequate for class use. The sections may be prepared by making use of sublimate-acetic for fixation, paraffin embedding, Delafield's hæmatoxylin and eosin as stains, and balsam for the mounting medium. For the sections of skin and intestine, the writer recommends Bouin's fixing fluid, and his own modification of Cajal's fuchsin and picro-indigo-carmine.[1] This gives a

[1] *Vertebrate Embryology*, page 283.

polychromatic stain which brings out the different layers most vividly.

Directions for the collection and preparation of frog eggs and embryos are given in the writer's *Vertebrate Embryology*, to which the reader is referred for further information (Chapter XI, *loc. cit.*)

MARINE BIOLOGICAL LABORATORY
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